

## CLAIMS.

1. A fluid container for containing a liquid nutrient having a  
5 pH equal to or greater than about 2.5, said container having an interior surface  
having a metal-ion sequestering agent for removing a designated metal ion from  
said liquid nutrient for inhibiting growth of microbes in said liquid nutrient.
2. A fluid container according to claim 1 wherein said metal-  
10 ion sequestering agent is immobilized on the surface(s) of said container and has a  
stability constant greater than  $10^{10}$  with iron (III).
3. A fluid container according to claim 1 wherein said  
sequestering agent is immobilized on the surface(s) of said container and has a  
15 high-affinity for biologically important metal ions such as Mn, Zn, Cu and Fe.
4. A fluid container according to claim 1 wherein said  
sequestering agent is immobilized on the surface(s) of said container and has a  
high-selectivity for biologically important metal ions such as Mn, Zn, Cu and Fe.  
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5. A fluid container according to claim 1 wherein said  
sequestering agent has a high-selectivity for certain metal ions but a low-affinity  
for at least one other ion.
- 25 6. A fluid container according to claim 5 wherein said certain  
metal ions comprises Mn, Zn, Cu and Fe and said other at least one ion comprises  
calcium.
7. A fluid container according to claim 1 wherein said metal-  
30 ion sequestering agent is immobilized on the surface(s) of said container and has a  
stability constant greater than  $10^{20}$  with iron (III).

8. A fluid container according to claim 1 wherein said metal-ion sequestering agent is immobilized on the surface(s) of said container and has a stability constant greater than  $10^{30}$  with iron (III).

5 9. A fluid container according to claim 1 wherein said metal-ion sequestering agent comprises derivatized nanoparticles comprising inorganic nanoparticles having an attached metal-ion sequestrant, wherein said inorganic nanoparticles have an average particle size of less than 200 nm and the derivatized nanoparticles have a stability constant greater than  $10^{10}$  with iron (III).

10 10. A fluid container according to claim 1 wherein said metal-ion sequestering agent is immobilized in a polymeric layer, and the polymeric layer contacts the fluid contained therein.

15 11. A fluid container according to claim 10 wherein the polymeric layer is permeable to water.

20 12. A fluid container according to claim 10 wherein the metal-ion sequestering agent comprises are 0.1 to 50.0 % by weight of the polymeric layer.

13. A fluid container according to claim 9 wherein said inorganic nanoparticles have an average particle size of less than 100 nm.

25 14. A fluid container according to claim 1 wherein said metal-ion sequestrant comprises an alpha amino carboxylate, a hydroxamate, or a catechol functional group.

30 15. A fluid container according to claim 1 wherein said metal-ion sequestrant comprises a naturally synthesized siderophore molecule.

16. A fluid container according to claim 9 wherein said metal-ion sequestrant is attached to the nanoparticle by reacting the nanoparticle with a silicon alkoxide intermediate of the sequestrant having the general formula:



wherein x is an integer from 1 to 3;

R is an alkyl group; and

R' is an organic group containing an alpha amino carboxylate, a hydroxamate, or  
10 a catechol.

17. A fluid container according to claim 1 further comprising a barrier layer wherein the polymeric layer is between the surface of the article and the barrier layer and wherein the barrier layer does not contain the derivatized  
15 nanoparticles.

18. A fluid container according to claim 17 wherein the barrier layer is permeable to water.

20 19. A fluid container according to claim 17 wherein the barrier layer has a thickness in the range of 0.1 microns to 10.0 microns.

20. A fluid container according to claim 17 wherein microbes cannot pass or diffuse through the barrier layer.

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21. A fluid container according to claim 1 wherein said container comprises a bottle.

22. A fluid container according to claim 20 wherein said bottle  
30 is made of a plastic material.

23. A fluid container according to claim 21 where a cap is provided with said bottle for sealing of said bottle.

24. A fluid container according to claim 1 where said liquid  
5 nutrient comprises a beverage.

25. A fluid container according to claim 1 wherein said container is in the form one of the following:

metal can,  
10 drink box,  
drink pouch,  
foil wrap,  
glass container,  
plastic container.

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26. A fluid container according to claim 1 wherein said sequestering agent is applied to the internal surface of said container.

27. A container according to claim 1 wherein sequestering  
20 agent is provided as an insert.

28. A fluid container according to claim 1 wherein said container is made of a material that includes said sequestering agent.

29. A fluid container according to claim 23 wherein said  
25 sequestering agent is provided on the internal surface of said cap.

30. A method for inhibiting growth of microbes in a liquid ,  
comprising the steps of;  
30 a. providing a container for holding a liquid having a pH equal  
to or greater than about 2.5, said container having an internal surface having an

ion sequestering agent provided on at least a portion of said internal surface for removing designated metal ions from said liquid;

b. filling said container with said liquid in an open environment;

5 c. closing said container with said liquid contained therein;  
and

c. shipping said container for use of said liquid without any further processing of said container containing said liquid.

10 31. A method according to claim 29 wherein said container is positioned so that said sequestering agent contacts said liquid.

32. A method according to claim 30 wherein said container comprises a bottle and cap assembly.

15 33. A method according to claim 32 wherein said bottle is made of a plastic material.

20 34. A method according to claim 30 wherein said sequestering agent is provided on the internal surface of said container.

35. A method according to claim 30 wherein said bottle is made of a material that includes said sequestering agent.

25 36. A method according to claim 32 wherein said sequestering agent is provided on the internal surface of said cap.

37. A method according to claim 30 wherein said liquid has a pH equal to or greater than about 4.

30 38. A method for bottling a liquid having a pH equal to or greater than about 2.5, comprising the steps of:

a. providing a container having a sequestering agent provided on at least a portion of said internal surface for inhibiting growth of microbes;

b. filling said container with a liquid having a PH equal to or greater than about 2.5; and

5 c. sealing said container with said liquid contained therein.

39. A method according to claim 38 wherein said container comprises a bottle and cap assembly.

10 40. A method according to claim 38 wherein sequestering agent in provided on the interior surface of said bottle.

41. A method according to claim 39 wherein sequestering agent in provided on the interior surface of said cap.

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42. A method according to claim 38 wherein sequestering agent in provided as an insert.

20 43. A method according to claim 38 wherein said bottle is made of a material that includes said sequestering agent.

44. A method according to claim 38 wherein said liquid is a beverage that is consumed by individuals.

25 45. A method according to claim 38 wherein said pH is equal to or greater than about 4.0.

30 46. An article for inhibiting the growth of a microbes in a liquid nutrient when placed in contact with the liquid nutrient, said article having a sequestering agent such that when said article is placed in contact with said liquid nutrient said sequestering agent inhibits the growth of microbes in said liquid nutrient, said sequestering agent comprising derivatized nanoparticles.

47. An article according to claim 46 wherein said derivatized nanoparticles comprise inorganic nanoparticles having an attached metal-ion sequestrant.

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48. An article according to claim 46 wherein said inorganic nanoparticles have an average particle size of less than 200 nm and the derivatized nanoparticles have a stability constant greater than  $10^{10}$  with iron (III).

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49. An article according to claim 46 wherein said sequestering agent is secured to said article by a support structure.

50. An article according to claim 49 wherein said sequestering agent is applied to the surface of said article that contacts said liquid nutrient.

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51. An article according to claim 50 wherein said support structure comprises a removable film layer.

52. A sealed fluid container for containing a liquid nutrient, said container having an interior surface having a metal-ion sequestering agent for removing a designated metal ion from said liquid nutrient for maintaining the level of microbes in said liquid nutrient below a predetermined amount.

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53. A sealed fluid container according to claim 52 wherein said predetermined amount comprises no pathogenous micro-organisms and a level of non harmful micro-organisms similar to the level after pasteurization during the shelf life of the product.

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54. A method of injection molding a multi-layer plastic preform to be blow molded into a container comprising the steps of:

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forming an inner layer of a preform of a first plastic material having an open end, sidewalls, and a closed end, said closed end having a plurality

of spaced channels on the exterior surface thereof extending from a central area on the closed end to the sidewalls, said first plastic material includes a sequestering agent for inhibiting the growth of microbes;

5 providing an overmolding mold cavity defined by sidewalls, an open end for accommodating a core rod, and a closed end with an injection gate there through, and a plurality of channels on the interior surface of the closed end communicating with the gate and extending from the gate to the sidewalls;

10 inserting a core rod and the inner plastic layer of the preform into said overmolding mold cavity with respective channels of the inner plastic layer of the preform and the overmolding mold cavity substantially aligned; and

overmolding a second layer of a second plastic material on the inner plastic layer of the preform to form an overmolded multi-layer perform in said overmolding mold cavity by flowing the second plastic material from the gate only through the respective channels to the sidewalls of the overmolding mold cavity thereby limiting the second material in the closed end of the multi-layer preform to regions of the channels,

15 providing internal pressure to said preform so as to form a container wherein the first plastic provides a surface for inhibiting the growth microbes with respect to a liquid placed in said container.

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55. The method of claim 54 wherein the first plastic comprises a hydrophilic polymer and said sequestering agent that is immobilized on the inner surface(s) of said container has a stability constant greater than  $10^{10}$  with iron (III).

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56. The method of claim 54 wherein the first plastic comprises hydrophilic polymer and said sequestering agent that is immobilized on the inner surface(s) of said container has a high-selectivity for biologically important metal ions such as Mn, Zn, Cu and Fe

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57. The method of claim 54 wherein sequestering agent comprises derivatized nanoparticles comprising inorganic nanoparticles having an



attached metal-ion sequestrant, wherein said inorganic nanoparticles have an average particle size of less than 200 nm and the derivatized nanoparticles have a stability constant greater than  $10^{10}$  with iron (III).

5                    58.     The method of claim 54 wherein the first plastic is PET and the second plastic is an injection moldable thermoplastic other than PET.

                  59.     A method of making a bottle having metal-ion sequestering agent, comprising the steps of:

10                    forming an inner layer of a plastic material having an open end, sidewalls, and a closed end, said closed end having a plurality of spaced channels on the exterior surface thereof extending from a central area on the closed end to the sidewalls, said plastic material includes a sequestering agent for inhibiting the growth microbes;

15                    providing an overmolding mold cavity defined by sidewalls, an open end for accommodating a core rod, and a closed end with an injection gate there through, and a plurality of channels on the interior surface of the closed end communicating with the gate and extending from the gate to the sidewalls;

                  inserting a core rod and the inner plastic layer of the preform into  
20                    said overmolding mold cavity with respective channels of the inner plastic layer of the preform and the overmolding mold cavity substantially aligned; and

                  providing internal pressure to said preform so as to form a container wherein the first plastic provides a surface for inhibiting the growth of microbes with respect to a liquid placed in said container.

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                  60.     The method of claim 59 wherein the first plastic material comprises a hydrophilic polymer and said sequestering agent that is immobilized on the surface(s) of said container has a stability constant greater than  $10^{10}$  with iron (III).

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                  61.     The method of claim 59 wherein the first plastic material comprises a hydrophilic polymer and said sequestering agent that is immobilized

on the surface(s) of said container has a high-selectivity for biologically important metal ions such as Mn, Zn, Cu and Fe

62. The method of claim 59 wherein the sequestering agent  
5 comprises derivatized nanoparticles comprising inorganic nanoparticles having an attached metal-ion sequestrant, wherein said inorganic nanoparticles have an average particle size of less than 200 nm and the derivatized nanoparticles have a stability constant greater than  $10^{10}$  with iron (III).

10 63. The method of claim 59 wherein the first plastic material is a hydrophilic polymer and the second plastic is an injection moldable thermoplastic.

64. A method of making a plastic container capable of  
15 inhibiting the growth of microbes in a liquid placed in said container, comprising the steps of:

- a. forming a plastic container having sidewall, an open end and a closed end that form a receiving chamber for holding a fluid, said chamber defining an interior surface of said container; and
- 20 b. applying a sequestering agent on said interior surface.

65. A method according to claim 64 wherein said sequestering agent is applied by spraying said sequestering agent on the surface of said container.

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66. The method according to claim 64 wherein sequestering agent is applied by coating the interior surface with a liquid.

67. The method according to claim 64 wherein said  
30 sequestering agent is immobilized on the surface(s) of said container and has a stability constant greater than  $10^{10}$  with iron (III).

68. The method container according to claim 64 wherein said sequestering agent is immobilized on the surface(s) of said container and has a high-affinity for biologically important metal ions such as Mn, Zn, Cu and Fe.

5 69. The method according to claim 64 wherein said sequestering agent is immobilized on the surface(s) of said container and has a high-selectivity for biologically important metal ions such as Mn, Zn, Cu and Fe.

70. The method according to claim 64 wherein said  
10 sequestering agent has a high-selectivity for certain metal ions but a low-affinity for at least other ions.

71. The method of claim 64 wherein said sequestering agent comprises derivatized nanoparticles comprising inorganic nanoparticles having an  
15 attached metal-ion sequestrant, wherein said inorganic nanoparticles have an average particle size of less than 200 nm and the derivatized nanoparticles have a stability constant greater than  $10^{10}$  with iron (III).

72. A method of making a sealed package capable of inhibiting  
20 the growth of microbes in a liquid placed in said container, comprising the steps of:

- a. forming said sealed container from at least one ply layer of a sheet material, said sheet material of one of said at least one ply layer having an interior side that will form the interior surface of said sealed package;
- 25 b. providing a sequestering agent on said interior side.

73. A method of making a sealed package according to claim 72 wherein at least one ply layer comprises a plurality of layers of sheet material.

30 74. A method of making a sealed package according to claim 72 wherein the said step of providing a sequestering agent comprises applying a coating said one of said at least one ply layers.

75. A method of making a sealed package according to claim 72 wherein the said step of providing a sequestering agent comprises forming said inner layer of said at least one ply layer of a material having said sequestering agent.

76. A bottle and cap assembly for containing a liquid nutrient having a pH equal to or greater than about 2.5, said bottle having an interior surface having a metal-ion sequestering agent for removing a designated metal ion from said liquid nutrient for inhibiting growth of microbes in said liquid nutrient.

77. A bottle and cap assembly according to claim 76 wherein said metal-ion sequestering agent is immobilized on the surface(s) of said bottle and has a stability constant greater than  $10^{10}$  with iron (III).

78. A bottle and cap assembly according to claim 76 wherein said sequestering agent is immobilized on the surface(s) of said bottle and has a high-affinity for biologically important metal ions such as Mn, Zn, Cu and Fe.

79. A bottle and cap assembly according to claim 76 wherein said sequestering agent is immobilized on the surface(s) of said bottle and has a high-selectivity for biologically important metal ions such as Mn, Zn, Cu and Fe.

80. A bottle and cap assembly according to claim 76 wherein said sequestering agent has a high-selectivity for certain metal ions but a low-affinity for at least one other ions.

81. A bottle and cap assembly according to claim 76 wherein said certain metal ions comprises Mn, Zn, Cu and Fe and said other at least one ion comprises calcium.

82. A bottle and cap assembly according to claim 76 wherein said metal-ion sequestering agent is immobilized on the surface(s) of said bottle and has a stability constant greater than  $10^{20}$  with iron (III).

5 83. A bottle and cap assembly according to claim 76 wherein said metal-ion sequestering agent is immobilized on the surface(s) of said bottle and has a stability constant greater than  $10^{30}$  with iron (III).

10 84. A bottle and cap assembly according to claim 76 wherein said metal-ion sequestering agent comprises derivatized nanoparticles comprising inorganic nanoparticles having an attached metal-ion sequestrant, wherein said inorganic nanoparticles have an average particle size of less than 200 nm and the derivatized nanoparticles have a stability constant greater than  $10^{10}$  with iron (III).

15 85. A bottle and cap assembly according to claim 76 wherein said metal-ion sequestering agent is immobilized in a polymeric layer, and the polymeric layer contacts the fluid contained therein.

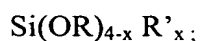
20 86. A bottle and cap assembly according to claim 76 wherein the polymeric layer is permeable to water.

25 87. A bottle and cap assembly according to claim 76 wherein the metal-ion sequestering agent comprises are 0.1 to 50.0 % by weight of the polymeric layer.

88. A bottle and cap assembly according to claim 76 wherein said inorganic nanoparticles have an average particle size of less than 100 nm.

30 89. A bottle and cap assembly according to claim 76 wherein said metal-ion sequestrant comprises an alpha amino carboxylate, a hydroxamate, or a catechol functional group.

90. A bottle and cap assembly according to claim 76 wherein said metal-ion sequestrant is attached to the nanoparticle by reacting the nanoparticle with a silicon alkoxide intermediate of the sequestrant having the general formula:



wherein x is an integer from 1 to 3;

R is an alkyl group; and

10 R' is an organic group containing an alpha amino carboxylate, a hydroxamate, or a catechol.

91. A bottle and cap assembly according to claim 76 wherein said bottle is made of a plastic material.

15 92. A bottle and cap assembly according to claim 76 wherein said liquid nutrient comprises a beverage.

93. A bottle and cap assembly according to claim 76 wherein  
20 said sequestering agent is applied to the internal surface of said bottle.

94. A bottle and cap assembly according to claim 76 wherein said bottle is made of a material that includes said sequestering agent.

25 95. A bottle and cap assembly according to claim 76 wherein said sequestering agent is provided on the internal surface of said cap.

96. A fluid container according to claim 1 wherein said sequestering agent is provided as an insert made of a material that includes said  
30 sequestering agent.

97. A fluid container according to claim 1 wherein said metal-ion sequestering agent comprises derivatized nanoparticles comprising inorganic nanoparticles having an attached bio-synthetic molecules such as a siderophore as the metal-ion sequestrant.